CROONIAN LECTURE.—"The Nature and Significance of Functional Metabolism in the Plant" (*Das Wesen und die Bedeutung des Betriebsstoffwechsels in der Pflanze*). By WILHELM PFEFFER, Sc.D. (Cantab.), of the University of Leipzig, For. Mem. R.S. Received March 9,—Read March 17, 1898.

(Translation of Author's Abstract.)

The fact that a mould fungus will thrive in a solution, from which, with the exception of certain inorganic acids, it can obtain nothing but sugar, affords proof that the elaboration of these food substances in metabolism not only provides the numerous carbon compounds which are concerned in the construction of the plant, but also serves as a sufficient source of energy for the performance of its functions. For in the plant, as in the animal, vital activity comes to a standstill if the conditions and the energy necessary for the discharge of its functions are not constantly provided by means of profound chemical decompositions. Just as in animals, a great amount of internal and external work has to be accomplished, in order to carry on and maintain the action of the organism. Hence the greater part, and in the mature plant even the whole, of the food absorbed is devoted to this functional metabolism, so that only a certain fraction of the sugar which has disappeared from the solution is to be found in the resulting crop of fungi, in the form of various carbon compounds. The rest of the sugar has been burnt up to form carbonic acid and water; that is to say, it has been sacrificed to the physiological combustion, which in these, as in most plants, is indispensable for gaining an adequate amount of kinetic energy.

But, just as man is able to obtain driving power, not only from the combustion of wood and coal, but also from the explosion of gunpowder or dynamite, so there are certain of the lower plants which gain their whole kinetic energy by means of chemical transformations and decompositions, which go on without the participation of free oxygen. Although the careful consideration of such organisms is indispensable for any correct estimate of functional metabolism, yet we may, in the first instance, limit our attention to oxygen-respiration, *i.e.*, to the functional metabolism of aërobic organisms.

In any case it is only after elaboration that food acquires its significance for the construction and working of the organism. With respect to the utilisation of the food, it is of no consequence whence it comes, or by what means the organism obtains it. Obvious as this consideration is, yet confusion between the elaboration of food in structural and functional metabolism on the one

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hand, and the operations adapted to the acquisition and absorption of organic nutriment on the other, has led to a grave error, namely, to the assumption that a difference in principle exists between the metabolism of plants and that of animals. The simple reflection that an immense number of plants exist which are destitute of chlorophyll, might at once have taught that the function of the chlorophyll apparatus—the production of food from carbonic acid and water—only serves to provide nutriment for further elaboration, and to introduce it in a peculiar and highly characteristic manner into the organism. For the structural and functional metabolism of green plants, however, the sugar prepared in the plant's own factory has exactly the same significance as the sugar which a fungus obtains from outside. In like manner it makes no difference to the utilisation and importance of sugar in the metabolism of man, whether a sugar-baker obtains it from his own factory or another man has to buy it at second or third hand.

Again, only those plants can dispense with a supply of albuminous food substances which construct these bodies synthetically from simpler compounds. In all plants, however, in close analogy with the animal organism, albuminous substances not only serve as permanent constituents of the body, but are in part again disintegrated in metabolism. Yet this process does not as a rule result in an excretion of the nitrogenous products of decomposition, for the latter are usually at once re-employed for the regeneration of albuminous substances. This, however, cannot take place to a sufficient extent in cases where a mould fungus is provided with protein substances as its only food, so as to increase the transformation of albuminoids and at the same time to restrict their regeneration. Under these circumstances a large amount of ammonium carbonate is actually excreted, in other words, the same final product which also arises in the animal body, but which there at once undergoes condensation to form urea. By this latter process the injurious effect which would result from an accumulation of ammonium carbonate is avoided. In the case of fungi such an injurious accumulation does not usually occur under normal conditions of growth, while these plants also to some extent possess the power of guarding against its deleterious influence by neutralisation, owing to the fact that in the presence of alkaline compounds oxalic acid is produced in increased quantity.

It constantly happens that all those processes which do not form an essential part of the indispensable functional metabolism, are regulated in such a manner as to be wholly or partly brought to a standstill without impairing other functions. Any excessive accumulation of products always has this result, so that, for example, the further formation of sugar or of protein bodies ceases, when these substances have collected in the cell up to a certain limited

amount. On the other hand, the inevitable final products of the general functional metabolism must be continually formed, for it is upon this chemical process that the maintenance of vital activity depends, and these final products, in so far as they are not again made use of, must also be constantly secreted and removed, for their accumulation would render further activity impossible. In many aërobic organisms, for reasons already indicated, only the excretion of carbonic acid and water is in question. In the case of many fungi, and some other plants, we find, however, in addition to these substances, organic acids, and other non-volatile final products, which are secreted in great variety and amount, especially in the case of many aërobic and anaërobic fermentations.

In order to avoid an accumulation in the cell, the final products which are continually arising, as well as the food to be assimilated, must necessarily be soluble and capable of diosmosis. Hence the ejection of the undigested remains of food is usually impossible, though, where it is possible, we find it in plants as well as in animals, as, for example, in the Myxomycetes. Extracellular digestion, which is employed on an extensive scale even in the vegetable kingdom, is, broadly speaking, only a means by which substances are rendered available for absorption and elaboration by the living elements, but is no more an integral part of the actual functional metabolism than is the digestion in the stomach of animals. The same holds good with reference to respiratory movements, and to all those operations and adaptations which provide for the access of oxygen and the removal of carbonic acid. In plants, it is true, there are no special active respiratory movements, but in all the larger plants an extensive system of aëration serves to maintain, adequately, the gaseous interchange of the internal cells. A loose combination of oxygen, such as is found in the hæmoglobin of the blood, is not of general occurrence among plants, though present and efficient in certain chromogenic bacteria.

If we leave out of consideration all subsidiary and preliminary processes, there is no doubt that the true aërobic functional metabolism is the same, in principle, in plants and in animals; in fact even from a formal point of view, no difference exists, if, as is fitting, we select the lowest animal and vegetable beings for comparison.

In plants, which like animals perform a large amount of work, vigorous respiration also takes place; in specially active plants it may even be actually greater than in warm-blooded animals. For while in man, the carbonic acid produced in twenty-four hours amounts to about 1·2 per cent., in many mould-fungi it exceeds 6 per cent. of the weight of the body; in very active bacteria the consumption of oxygen, referred to the same standard, may reach an amount 200 times as great as in man.

Although such energetic physiological combustion involves a very considerable production of heat, yet in consequence of the extensive radiating surface, only a slight rise of temperature usually ensues. The functional metabolism, in fact, as in pœcilothermic organisms, does not provide for the regulative maintenance of a definite body-temperature. At the same time the plant is adapted to accommodate itself to temperatures, ranging for example, from 2° to 40° C., and to bear such oscillations of the body-temperature without injury. Manifestly it is altogether expedient that, when the temperature rises, the activity of growth and respiration should be simultaneously accelerated. On the other hand, the fact that when the optimum temperature for growth has been exceeded, substances are burnt up by respiration, in an ever increasing degree, while the processes of growth and movement are retarded or altogether stopped, is a non-adaptive phenomenon, determined only by the absence of any regulative check.

As long as the external conditions remain constant, however, respiration is always regulated by the plant, and is in general increased, as the activity of the whole organism automatically rises. This happens, for example, when a plant begins to grow again, after completing the winter's rest, or when a traumatic reaction is called forth in consequence of injury. Thus, if we cut a potato in pieces, the production of carbonic acid gradually increases nine- or ten-fold, in the course of twenty-four hours, owing to the respiratory process, and then gradually diminishes again as the traumatic reaction passes off. Here the plant falls as it were into a state of fever, for simultaneously with respiration, the production of heat is very considerably augmented.

If we bear in mind that the essential office of functional metabolism, consists in providing, by means of chemical transformations, the necessary energy for vital action, we cannot be surprised that this end is not always attained in the same way. Apart from the fact that different carbon-compounds are consumed in respiration, and that not only carbonic acid, but in certain plants oxalic acid, acetic acid, citric acid, &c., arise as the final products of physiological combustion, there exist even aërobic organisms in which kinetic energy is no longer obtained by the oxidation of any carbon compounds whatever. Among these are the nitrobacteria, some of which oxidise ammonia to nitrous acid, while others oxidise nitrous to nitric acid; these remarkable organisms, with the help of the energy thus gained, are at the same time capable of constructing their organic food synthetically from carbonic acid. Again, in the respiratory process of the sulphur-bacteria, the sulphuretted hydrogen undergoes combustion, sulphur being first set free, and then oxidized to form sulphuric acid. Thus, in these organisms, sulphuric acid is secreted as

the final product of physiological combustion, while in the nitro-bacteria the same is the case with nitrous or with nitric acid. There can be no question that as time goes on, yet other specific peculiarities will be discovered. Thus it is conceivable that certain micro-organisms may gain their chemical kinetic energy by the oxidation of ferrous oxide, and others perhaps, by the oxidation of hydrogen or of the gaseous hydrocarbons.

Considered from the general point of view of energy, it is by no means necessary that physiological combustion should proceed along the same lines in all organisms. Indeed it was only in consequence of an unjustifiable generalisation from observations on the higher animal and vegetable organisms, that the belief arose that organic life is impossible without the agency of free oxygen, *i.e.*, without oxygen-respiration. Just, however, as man is able to employ driving power, derived from such reactions as the explosion of gunpowder or dynamite in a space free from oxygen, so must it appear *à priori* possible, that organisms have been evolved on our earth, in adaptation to special conditions and necessities of life, which are able to live without making use of free oxygen.

As a matter of fact numerous anaërobic micro-organisms are now known. Their existence, indeed, was established as long ago as 1861, by the investigations of Pasteur, and it was only the deeply rooted belief in the absolute indispensability of oxygen-respiration which caused the majority of the learned to remain sceptical, or to endeavour to save the dogma of the necessity of oxygen by forced, and often, frivolous interpretations.

Much, it is true, remains to be explained as to the details of metabolism, both in aërobic and anaërobic organisms; meanwhile this at least is certain, that even in anaërobes, kinetic energy is gained by means of a great variety of chemical transformations. This fact is at once indicated by the various final products of functional metabolism, in one organism consisting chiefly of alcohol and carbonic acid, in another of butyric acid, lactic acid, or butyl-alcohol, or of other very various volatile and non-volatile compounds, which, however, often owe their origin in part to secondary processes; considered from the standpoint of energy it is not necessary that any gaseous products should arise, or even that oxygen atoms should be transposed, or carbon compounds disintegrated. It is true that the latter assumption holds good in all cases which have as yet been minutely studied, but it is quite conceivable that kinetic energy may be obtained by some other reaction (for example, by the reaction between potassium nitrate and sulphur).

The anaërobia are, however, of great importance in the economy of nature, for by their agency decomposition is carried on in the interior of the cadaver, and generally in places where the conditions

for oxygen-respiration are absent. Thus, in the case of facultative anaërobes, in proportion as oxygen becomes deficient, aërobic becomes replaced by anaërobic metabolism, while the obligatory anaërobes now for the first time begin their growth and multiplication. This is correlated with the fact, that oxygen, even at low tension, acts as a poison to them, and when air is so much compressed that the oxygen is twenty or thirty times as dense as in the atmosphere, all plants perish. All gradations of sensitiveness towards oxygen occur, from the most resistant organisms downwards, and there are even obligatory aërobes, such as the sulphur-bacteria, which can only exist when oxygen is of very low density.

Thus the different types are connected by intermediate links. For any facultative anaërobe it is possible to prepare a nutritive substratum on which it can flourish only when able to respire free oxygen. If, however, the oxygen be presented in sufficient dilution it does not hinder the growth of the anaërobic organism, which, under these circumstances, constantly draws the free oxygen into its functional metabolism, and thus gradually consumes it in considerable quantities. Further, a variable density of the free oxygen may be endured by the same organism in accordance with the cultural conditions under which it is placed. It has in fact been found possible, by means of special nutrition, to cultivate the strictly anaërobic Bacillus of symptomatic anthrax (*B. carbonis*) as an aërobe. The habits of the nitrogen-assimilating *Clostridium Pasteurianum* are also evidently modified by certain bacteria which usually live associated with it, for in such symbiosis *Clostridium* endures the free access of air, while in the isolated condition it can only live anaërobically.

Even in the case of typical aërobes, however, the withdrawal of oxygen does not bring the metabolic activity entirely to a standstill. At first carbonic acid is still given off, being derived from the intramolecular respiration, *i.e.*, from chemico-physiological processes, which in most plants further result in the formation of alcohol and other products. This intramolecular respiration is thus a vital action which is of importance for the maintenance of life even in aërobes, but which in the latter does not suffice to maintain the whole working of the organism after oxygen has been withdrawn. In the anaërobes this capacity has been fully developed, but of course only comes into play when suitable nutrition is provided. For, when differently fed, even the facultative anaërobe can only grow if fully supplied with air, and behaves, when oxygen is withdrawn, just like a typically aërobic plant, for it then ceases to grow, and, sooner or later, perishes altogether. By means of an appropriate food-mixture, however, we can ensure that the facultative anaërobe continues its growth and movements, but only for a certain time and to a certain extent, thus appearing as a temporarily anaërobic organism. Such

an organism, for example, is yeast (*Saccharomyces cerevisiæ*), which, under the nutritive conditions hitherto tested, cannot live without an occasional supply of oxygen, whereas certain bacteria are capable of an unlimited anaërobic life.

It follows directly from what has been said, that among aërobes life is maintained for a limited time by the action of intramolecular respiration. For it is only when intramolecular respiration fails that the plant suffers in an atmosphere free from oxygen; the strictly aërobic mould-fungi keep alive much longer without oxygen, when their aërobic respiratory activity is intensified by providing them with sugar. Thus there is every gradation and transition between those organisms which require free oxygen, and those in which the anaërobic metabolic activity, which is exercised to a certain degree in all organisms, is so far developed and utilised that the functional metabolism suffices for a life without oxygen. Even in aërobes a number of partial functions are carried on for a certain time after the withdrawal of oxygen. Among these functions intramolecular respiration itself is included, as well as all the metabolic changes with which it is linked. There are also certain processes of growth and movement which are not at once brought to a standstill when oxygen is withdrawn. Thus we know that nuclear division, when it has once begun, still goes on in the absence of oxygen, and under the same conditions the tentacles of the insectivorous Sundew still carry out their movements when stimulated. The muscle of animals can also be caused to contract when deprived of oxygen.

The relations of the organism to its conditions of life can be demonstrated and understood, even though we do not possess any deeper insight into the causes and the exact processes of functional metabolism. We may also regard it as certain that functional metabolism is indispensable to vital activity, on which in its turn it depends and by which it is regulated, so that metabolism is at once extinguished when death ensues. Thus the realisation of functional metabolism ensures the continuity of metabolism in general, just as a blazing fire, by heating the wood, constantly creates and maintains the conditions necessary for the continuance of combustion.

It is also certain that functional metabolism runs its course within the living protoplasm, not merely on its surface or in particular portions of it, but in and between all its constituent parts, as must necessarily be the case in order that vital activity may be maintained. This can be seen at once from the fact that those movements in the protoplasm, or in any separate fragment of the protoplast, which are dependent on aërobic respiration, come to an end on the withdrawal of oxygen, even when the adjoining cells have access to oxygen and are in a state of full activity.

From the dependence of functional metabolism on vital activity it

directly follows that the consumption of the material to be elaborated, as well as the absorption of free oxygen by the organism, is regulated in accordance with its requirements. Consequently, when these requirements are fully satisfied, an increased supply of food material or of oxygen results in no essential acceleration of the functional metabolism. For this reason plants do not breathe any more vigorously in pure oxygen than in ordinary air, for even in the latter much more oxygen penetrates into the cell than is consumed in normal respiration.

If, however, the supply is not sufficient to fully satisfy the demand, then the functional metabolism, and with it the whole activity of the organism, is unavoidably reduced, just as a fire can no longer burn properly when insufficiently supplied with fuel or with oxygen. Most plants, however, can completely meet their demand for oxygen in an atmosphere in which the proportion of oxygen (at ordinary atmospheric pressure) is reduced to 5—8 per cent., so that on the highest mountains vegetable organisms find a more than sufficient density of oxygen. If its density be still further diminished, then, after a transient disturbance, the respiration and the total activity of the plant are depressed, so that in an atmosphere containing only 2—4 per cent. of oxygen the plant, though it survives, breathes and works in a diminished degree.

Since functional metabolism depends on the vital activities, a satisfactory causal explanation of the former will only be possible after we have gained a sufficient insight into the latter. In general, however, we may say that the same processes which effect intramolecular respiration also develop those affinities, by means of which free oxygen, when supplied, is drawn into metabolism. For intramolecular respiration is at once stopped on access of oxygen, and after the withdrawal of oxygen is immediately resumed. In much the same way as the development of the spontaneously inflammable phosphuretted hydrogen brings about the fixation of a certain amount of oxygen, may physiological combustion be determined and regulated through the continual formation of a single autoxydable body. In respiration, however, we have evidently to do with complicated reactions and reciprocal changes, which come into play between the constituent parts of the protoplasmic body. And further, the respiratory process must take a somewhat different form when, instead of a carbon compound, ammonia or ammonium nitrite, sulphuretted hydrogen or sulphur, forms the material for physiological combustion.

We may be sure, however, that in the plant passive oxygen is drawn into metabolism, and that oxygen is not brought into the active state in order to accomplish physiological oxidation. For we can prove with complete certainty that at no time does any such

process of oxidation occur in the interior of vitally active protoplasm (including the nucleus), as is brought about by even the feeblest form of active oxygen (hydrogen peroxide). This still holds good even if the reactions of active oxygen are obtained in the expressed sap, *i.e.*, after mixing bodies which in the plant are separate. If, however, active oxygen ever plays any part at all, it is at most to be regarded as one only of the means of which the organism avails itself, and not as revealing the true and essential cause of functional metabolism.

Side by side with the general process of functional metabolism, many other chemical operations must necessarily come into play, in order to provide the various compounds which are formed in the organism, in order to build up its tissues, or otherwise. Although these processes are not of necessity in continuous action, it is difficult to separate them from the general functional metabolism. We are placed with regard to the plant, somewhat in the position of a man who, while he can control the raw material introduced into a factory and the finished products turned out from it, is not permitted to inspect the internal working. Unless the observer has a knowledge of this from other sources, it is simply impossible for him to say what is the nature of all the manifold operations carried on in a chemical factory, whether simultaneously or successively, jointly or separately. At the same time the observer may be quite aware that all work in the factory is impossible if the fire be not burning under the boiler, or if the driving power in general be not available, and he may also know that the gaseous products of combustion, the ashes and the slag, must be got rid of, simply in order to make room for the work to go on. In the factory, however, just as in the plant, the general driving power is not always utilised for the same purposes or with equal efficiency. Indeed, when the steam-engine is at work but the rest of the machinery is out of gear, the whole driving power is wasted. No less is it true, in the case of the plant, that the relation between the available kinetic energy and its utilisation for various purposes, or in other words the economic coefficient, may vary within very wide limits according to the stage of development and the external conditions.